

We Claim:

1. Isolated nucleic acid molecule which codes for tumor rejection antigen precursor or is complementary to a nucleic acid molecule which codes for tumor rejection antigen precursor.
2. The isolated nucleic acid molecule of claim 1, wherein said molecule codes for a tumor rejection antigen precursor.
3. Isolated nucleic acid molecule of claim 1, wherein said molecule codes for a human tumor rejection antigen precursor.
4. The isolated nucleic acid molecule of claim 1, wherein said molecule is complementary to a nucleic acid molecule which codes for tumor rejection antigen precursor.
5. The isolated nucleic acid molecule of claim 1, wherein said molecule is DNA.
6. The isolated nucleic acid molecule of claim 1, wherein said molecule is RNA.
7. The isolated nucleic acid molecule of claim 1, wherein said molecule is a gene.

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8. The isolated nucleic acid molecule of claim 5, wherein said DNA is genomic DNA.

9. The isolated nucleic acid molecule of claim 5, wherein said DNA is cDNA.

10. The isolated nucleic acid molecule of claim 6, wherein said RNA is mRNA.

11. The isolated nucleic acid molecule of claim 4, wherein said molecule hybridizes to isolated nucleic acid which codes for tumor rejection antigen precursor under stringent conditions.

12. *An* The isolated nucleic acid molecule of claim 1, wherein said molecule codes for *the tumor rejection antigen* *which is complementary to a molecule which codes for a MAGE antigen.*

13. The isolated nucleic acid molecule of claim 12, wherein said MAGE antigen is selected from the group consisting of mage 1, mage 2, mage 3, mage 4, mage 5, mage 6, and mage 7.

14. The isolated nucleic acid molecule of claim 12, wherein said molecule codes for a MAGE antigen.

15. The isolated nucleic acid molecule of claim 12, wherein said molecule is complementary to a molecule which codes for a MAGE antigen.
16. The isolated nucleic acid molecule of claim 12, wherein said molecule is DNA.
17. The isolated nucleic acid molecule of claim 12, wherein said molecule is RNA.
18. The isolated nucleic acid molecule of claim 12, wherein said molecule is a gene.
19. The isolated nucleic acid molecule of claim 16, wherein said DNA is genomic DNA.
20. The isolated nucleic acid molecule of claim 16, wherein said nucleic acid molecule ¹₂
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which hybridizes to the molecule which codes for a MAGE antigen under stringent conditions.

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Nucleic acid molecule of claim 12

~~23. The isolated nucleic acid molecule of claim 13, wherein said molecule hybridizes to a molecule which codes for a MAGE antigen under stringent conditions.~~

24. Isolated nucleic acid molecule of claim 1, coding for tumor rejection antigen precursor for mastocytoma.

25. Isolated nucleic acid molecule of claim 1, coding for tumor rejection antigen precursor P815.

26. Isolated nucleic acid molecule of claim 1, having the nucleotide sequence of figure 6.

27. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 2.

~~28. Biologically pure culture of a ^{host} cell line transfected with the nucleic acid sequence of claim 12.~~

29. Biologically pure culture of a cell line transfected with the nucleic acid sequence of claim 22.

30. Biologically pure culture of a cell line of claim 27, selected from the group consisting of P1A.T2 and P1A.TC3.1.

16 The host

35. Biologically pure cell line of claim 34, said tumor rejection antigen precursor is MAGE1 and said isolated DNA has nucleic acid sequence: ¹⁵ ~~wherein~~ ⁴⁴⁸ ~~the~~ ¹ ~~as follows after sequence~~

10 20 30 40 50 60

1 GGATCCAGGC CCTGCCAGGA AAAATATAAG GGCCTGCGT GAGAACAGAG GGGGTCACTCC 60
 61 ACTGCATGAG AGTGGGGATG TCACAGAGTC CAGCCCCACCC TCCCTGGTAGC ACTGAGAAGC 120
 121 CAGGGCTGTG CTGCGGTCT GCACCCCTGAG GGGCCGTGGA TTCCCTTTCC TGGAGCTCCA 180
 181 GGAACCAAGGC AGTGAGGCTC TGGTCTGAGA CAGTATCCTC AGGTCAACAGA GCAGAGGATG 240
 241 CACAGGGTGT GCCAGCAGTG AATGTTTGCC CTGAATGAC ACCAAGGGCC CCACCTGCCA 300
 301 CAGGACACAT AGGACTCCAC AGAGTCCTGC CTCACCTCCC TACTGTCACT CCTGTAGAAT 360
 361 CGACCTCTGC TGGCCGGCTG TACCCCTGAGT ACCCTCTCAC TTCCCTCCCTC AGGTTTCAG 420
 421 GGGACAGGCC AACCCAGAGG ACAGGATTCC CTGGAGGCCA CAGAGGAGCA CCAAGGAGAA 480
 481 GATCTGTAAG TAGGCCCTTG TTAGAGTCCTC CAAGGTTCAAG TTCTCAGCTG AGGCCTCTCA 540
 541 CACACTCCCT CTCTCCCCAG GCCTGTGGGT CTTCAATTGCC CAGCTCCTGC CCACACTCCT 600
 601 GCCTGCTGCC CTGACGGAGAG TCATCATGTC TCTTGAGCAG AGGAGTCTGC ACTGCAAGCC 660
 661 TGAGGAAGCC CTTGAGGCCA AACAAAGAGC CCTGGGGCTGG TGTGTGTGCA GGCTGCCACC 720
 721 TCCCTCTCCT CTCCCTCTGGT CCTGGGCCACC CTGGAGGAGG TGGCCACTGC TGGGTCAACA 780
 781 GATCCTCCCCC AGAGTCCTCA GGGAGCCTCC GCCTTTCCCA CTACCATCAA CTTCACTCGA 840
 841 CAGAGGCAAC CCAGTGAGGG TTCCAGCAGC CGTGAAGAGG AGGGGCCAAG CACCTCTTGT 900
 901 ATCCTGGAGT CCTTGTTCCG AGCACTAATC ACTAAGAAGG TGGCTGATTT GGTTGGTTTT 960
 961 CTGCTCCTCA AAATATCGAGC CAGGGAGCCA GTCACAAAGG CAGAAATGCT GGAGAGTGTG 1020
 1021 ATCAAAATT ACAAGCACTG TTTTCTGAG ATCTTCGGCA AAGCCTCTGA GTCCCTGCAG 1080
 1081 CTGGTCTTTG GCATTGACGT GAAGGAAGCA GACCCCCACCG GOCACCTCCIA TGTCCTTGTC 1140
 1141 ACCTGCCTAG GTCTCTCTCA TGATGGCCTG CTGGGTGATA ATCAAGATCAT GCCCAGAGCA 1200
 1201 GGCTTCTGA TAATTGTCTT GGTATGATT GCAATGGAGG GCGGCCATGC TCCGTAGGAG 1260
 1261 GAAATCTGGG AGGAGCTGAG TGTGATGGAG GTGTATGATG GGAGGGAGCA CAGTGCCTAT 1320
 1321 GGGGAGGCCA GGAAAGCTGCT CACCCAAAGAT TTGGTGCAGG AAAAGTACCT GGAGTACGGC 1380
 1381 AGGTGCCGGGA CAGTGATCCC GCACGCTATG 1440
 1441 AAACCAAGCTA TGTGAAAGTC CTTGAGTATG TGATCAAGGT CAGTGCAAGA GTTCGTTTT 1500
 1501 TCTTCCCCATC CCTGCGTGAA GCAGCTTTGA GAGAGGAGGA AGAGGGAGTC TGAGCATGAG 1560
 1561 TTGCAGGCCA GGCCAGTGAGG AGGGGGACTG GGCAGTGCA CCTTCCAGGG CGCGGTCCAG 1620
 1621 CAGCTTCCCC TGCCCTCGTGT GACATGAGGC CCATTCTCA CTCTGAAAGAG AGCGGTCACT 1680
 1681 GTTCTCAGTA GTAGGTTCT GTTCTATTGG GTGACTTGGA GATTATCTT TGTTCTCTT 1740
 1741 TGGAAATTGTT CAAATGTTT TTTTAAAGGG ATGGTTGAAT GAACTTCAGC ATCCAAGTTT 1800
 1801 ATGAATGACA GCAGTCACAC AGTTCTGTGT ATATAGTTA AGGGTAAGAG TCTTGTGTTT 1860
 1861 TAATTCAAGTT GGGAAATCCA TTCTATTGG TGAAATTGGGA TAATAACAGC AGTGGAAATAA 1920
 1921 GTACTTAAAGA ATGTGAAAAA TGAGCAGTAA AATAGATGAG ATAAAGAACT AAAGAAATTA 1980
 1981 AGAGATAGTC AATTCTTGCC TTATACCTCA GTCTATTCTG TAAAATTTT AAAGATATAT 2040
 2041 GCATACCTGG ATTCTCTTGG CTTCTTGAG ATGTAAAGAG AAATTAATC TGAAATAAGA 2100
 2101 ATCTCTCTG TTCACTGGCT CTTTCTCTC CCATGCACTG AGCATCTGCT TTTTGGAGG 2160
 2161 CCCTGGGTAA GTAGTGGAGA TGCTAAAGGT AGCCAGACTC ATACCCACCC ATAGGGTCGT 2220
 2221 AGAGTCTAGG AGCTGCACTC ACGTAATCGA GGTGGCAAGA TGTCTCTAA AGATGTAGGG 2280
 2281 AAAAGTGAGA GAGGGGTGAG GGTGTGGGGC TCCGGGTGAG AGTGGTGGAG TGTCAATGCC 2340
 2341 CTGAGCTGGG GCATTTGGG CTTGGAGAA CTGCACTTCC TTCTGGGGGA GCTGATTGTA 2400
 2401 ATGATCTGG GTGGATCC 2418

10 20 30 40 50 60

36. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence coding for a cytokine.
37. The biologically pure culture of claim 36, wherein said cytokine is an interleukin.
38. The biologically pure culture of claim 37, wherein said interleukin is IL-2.
39. The biologically pure culture of claim 37, wherein said interleukin is IL-4.
40. The biologically pure culture of claim 27, wherein said cell line is transfected by a nucleic acid sequence which codes for an MHC molecule or an HLA molecule.
41. The biologically pure culture of claim 27, wherein said cell line expresses an MHC or HLA molecule which presents a tumor rejection antigen derived from a tumor rejection antigen precursor (TRAP), wherein said TRAP is coded for by a nucleic acid sequence transfected into said cell line.
42. The biologically pure culture of claim 27, wherein said culture is a mammalian cell culture and is non-proliferative.

13 *first cell*12
42

43. The ~~biologically pure culture~~ of claim 27, wherein said cell line is a fibroblast cell line.

14 *A expression*

44. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 2 operably linked to a promoter.

45. Expression vector of claim 44, wherein said promoter is a strong promoter.

6 *The expression*

46. Expression vector of claim 44, wherein said promoter is ~~a~~ 5
an inducible differential promoter.

47. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 7 operably linked to a promoter.

48. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 13 operably linked to a promoter.

49. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 14 operably linked to a promoter.

increase in said parameter is indicative of said cancerous condition.

141. Method of claim 140, wherein said factor is tumor necrosis factor.

142. Method for following progress of a therapeutic regime designed to alleviate a cancerous condition, comprising:

(a) assaying a sample from a subject to determine level of a parameter selected from the group consisting of (i) tumor rejection antigen, (ii) a cytolytic T cell specific for cells presenting said tumor rejection antigen, and (iii) an antibody which specifically binds to said tumor rejection antigen at a first time period;

(b) assaying level of the parameter selected in (a) at a second period of time and comparing it to the level determined in (a) as a determination of effect of said therapeutic regime.

add
85

138. Method for treating a subject afflicted with a cancerous condition, comprising:

- (i) identifying cells from said subject which express a tumor rejection antigen precursor and present a tumor rejection antigen derived from said precursor on their surface;
- (ii) isolating a sample of said cells;
- (iii) cultivating said cell, and;
- (iv) introducing said cells to said subject in an amount sufficient to provoke an immune response against said cells.

139. Method of claim 138, further comprising rendering said cells non proliferative, prior to introducing them to said subject.

140. Method for identifying a cytotoxic T cell useful in treating a subject afflicted with a cancerous condition, comprising:

- (i) identifying a tumor rejection antigen presented by cells associated with said cancerous condition derived from a tumor rejection antigen precursor expressed by said cells, prior to introducing them to said subject;
- (ii) contacting a cell presenting said antigen to a cytotoxic T cell, and;
- (iii) measuring a parameter selected from the group consisting of (i) proliferation of said cytotoxic T cell and (ii) release of a cytotoxic T cell produced factor, wherein

133. Method of claim 130, wherein said human leukocyte antigen is HLA-A1.

134. Method of claim 132, wherein said interleukin is IL-2.

135. Method of claim 132, wherein said interleukin is IL-4.

136. Method for treating a subject afflicted with a cancerous condition comprising administering to said subject an amount of a reagent consisting essentially of non-proliferative cell having expressed on its surface a tumor rejection antigen characteristic of cancerous cells in an amount sufficient to elicit an immune response thereto.

137. Method for treating a subject afflicted with a cancerous condition comprising administering to said patient an antibody which specifically binds to a tumor rejection antigen expressed on a cancer cell associated with said condition, said antibody being coupled to an anticancer agent, in an amount sufficient to treat said cancerous condition.

128. Method of claim 127, further comprising treating said cell to render it non-proliferative.

129. Method for preparing a biological material useful in treating a subject afflicted with a cancerous condition, comprising:

(i) transfecting a host cell with a nucleic acid molecule which codes for or expresses a tumor rejection antigen precursor;

(ii) transfecting said host cell with a nucleic acid molecule which codes for human leukocyte antigen which presents a tumor rejection antigen derived from said tumor rejection antigen precursor on a cell surface, and;

(iii) treating said host cells under conditions favoring expression of said nucleic acid molecules, and presentation of said tumor rejection antigen by said human leukocyte antigen.

130. Method of claim 129, further comprising treating said host cells to render them non proliferative following presentation of said tumor rejection antigen.

131. Method of claim 130, further comprising transfecting said host cell with a nucleic acid molecule which codes for or expresses a cytokine.

132. Method of claim 131, wherein said cytokine is an interleukin.

124. Method of claim 123, further comprising treating said cells to render them non-proliferative.

125. Method for treating a subject with a cancerous condition, comprising:

- (i) identifying a MAGE gene expressed by said tumor;
- (ii) transfecting a host cell having the same HLA type as said patient with said MAGE gene;
- (iii) culturing said transfected cells to express said MAGE gene, and;
- (iv) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

126. Method of claim 125, further comprising treating said cells to render them non proliferative.

127. Method for treating a subject with a cancerous condition, comprising administering to said subject an amount of a cell transfected with (i) a nucleic acid sequence which codes for a tumor rejection antigen precursor (TRAP) and (ii) a nucleic acid sequence which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said TRAP, wherein said tumor rejection antigen is presented by cells associated with said cancerous condition sufficient to alleviate said cancerous condition.

119. Method for treating a subject afflicted with a cancerous condition, comprising:

- (i) identifying a MAGE gene expressed by cancer cells associated with said condition;
- (ii) identifying an HLA molecule which presents a portion of an expression product of said MAGE gene;
- (iii) transfecting a host cell having the same HLA molecule as identified in (ii) with said MAGE gene;
- (iv) culturing said transfected cells to express said MAGE-gene, and;
- (v) introducing an amount of said cells to said subject sufficient to provoke an immune response against said tumor.

120. Method of claim 119, wherein said immune response comprises a B-cell response.

121. Method of claim 119, wherein said immune response is a T-cell response.

122. Method of claim 120, wherein said B cell response comprises production of antibodies specific to said tumor rejection antigen or tumor rejection antigen precursor.

123. Method of claim 121, wherein said T-cell response comprises generation of cytolytic T-cells specific for cells presenting said tumor rejection antigen.

115. Method of claim 108, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said tumor rejection antigen precursor.

116. Method of claim 108, comprising assaying said sample for shed tumor rejection antigen.

117. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for a cytolytic T cell specific for a tumor rejection antigen, presence of said cytolytic T cell being indicative of said cancerous condition.

118. Method for treating a subject afflicted with cancerous condition, comprising:

(i) removing a lymphocyte containing sample from said subject,

(ii) contacting the lymphocyte containing sample to a cell line transfected with a gene coding for an expressing a gene for a tumor rejection antigen precursor expressed by cancer cells associated with said conditions, under conditions favoring production of cytotoxic T cells against a tumor rejection antigen derived from said tumor rejection antigen precursor, and

(iii) introducing said cytotoxic T cells to said subject in an amount sufficient to lyse said cells.

108. Method for determining regression or progression of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) tumor rejection antigen precursor, (ii) tumor rejection antigen and (iii) cytolytic T cells specific for a tumor rejection antigen associated with said cancerous condition, wherein amount of said parameter is indicative of progression or regression of said cancerous condition.

109. Method of claim 108, wherein said sample is a body fluid.

110. Method of claim 108, wherein said sample is a tissue.

111. Method of claim 108, comprising contacting said sample with an antibody which specifically binds with said tumor rejection antigen or tumor rejection antigen precursor.

112. Method of claim 111, wherein said antibody is a monoclonal antibody.

113. Method of claim 108, comprising amplifying RNA which codes for said tumor rejection antigen precursor.

114. Method of claim 113, wherein said amplifying comprises carrying out polymerase chain reaction.

103. Antibody of claim 102, wherein said antibody is a monoclonal antibody.

104. Antibody of claim 98, wherein said tumor rejection antigen is antigen F.

105. Antibody of claim 104, wherein said antibody is a monoclonal antibody.

106. Method for diagnosing a cancerous condition in a subject, comprising contacting a lymphocyte containing sample of said subject to a cell line transfected with a DNA sequence coding for a tumor rejection antigen precursor expressed by cells associated with said cancerous condition, and determining lysis of said transfected cell line by a cytotoxic T cell line specific for a tumor rejection antigen derived from said tumor rejection antigen precursor, said lysis being indicative of said cancerous condition.

107. Method of claim 106, wherein said tumor rejection antigen precursor is a MAGE molecule.

94. Antibody of claim 88, wherein said tumor rejection antigen precursor is a MAGE precursor.
95. Antibody of claim 94, wherein said antibody is a monoclonal antibody.
96. Antibody of claim 94, wherein said MAGE precursor is mage 1, mage 2, mage 3, mage 4, mage 5, mage 6 and mage 7.
97. Antibody of claim 96, wherein said antibody is a monoclonal antibody.
98. Antibody which specifically binds to a tumor rejection antigen.
99. Antibody of claim 98, wherein said antibody is a monoclonal antibody.
100. Antibody of claim 98, wherein said tumor rejection antigen is that set forth in figure 10.
101. Antibody of claim 100, wherein said antibody is a monoclonal antibody.
102. Antibody of claim 98, wherein said tumor rejection antigen is antigen E.

80. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on a tumor rejection antigen precursor specific for a tumor characteristic of said cancerous condition, and a pharmaceutically acceptable carrier.
81. Composition of matter of claim 80, wherein said cell line is a human cell line.
82. Composition of matter of claim 80, wherein said pharmaceutically acceptable carrier is a liposome.
83. Composition of matter useful in treating a cancerous condition comprising a non proliferative cell line having expressed on its surface a tumor rejection antigen specific for a tumor characteristic of said cancerous condition, and a pharmaceutically acceptable carrier.
84. Composition of matter of claim 83, wherein said cell line is a human cell line.
85. Composition of matter of claim 83, wherein said pharmaceutically acceptable carrier is a liposome.

86. Composition of matter useful in treating a cancerous condition, comprising (i) a tumor rejection antigen or tumor rejection antigen precursor, (ii) an MHC or HLA molecule, and (iii) a pharmaceutically acceptable carrier.

87. Composition of matter of claim 86, wherein said pharmaceutically acceptable carrier is a liposome.

88. Antibody which specifically binds to a tumor rejection antigen precursor.

89. Antibody of claim 88, wherein said antibody is a monoclonal antibody.

90. Antibody of claim 88, wherein said tumor rejection antigen precursor is mage-1.

91. Antibody of claim 90, wherein said antibody is a monoclonal antibody.

92. Antibody of claim 88, wherein said tumor rejection antigen precursor is antigen F precursor.

93. Antibody of claim 92, wherein said antibody is a monoclonal antibody.

73. Vaccine of claim 72 wherein said precursor is mage-1.

74. Vaccine of claim 72, wherein said precursor is antigen F precursor.

75. Vaccine useful in treating a patient with a cancer comprising a tumor rejection antigen of claim 66 which provokes an immune response when administered to a subject.

76. Vaccine of claim 75, wherein said tumor rejection antigen has amino acid sequence of figure 10.

77. The vaccine of claim 75, wherein said tumor rejection antigen is antigen E.

78. The vaccine of claim 75, wherein said tumor rejection antigen is antigen F.

79. Isolated peptide useful in treating a subject afflicted with a cancerous condition, said peptide having amino acid sequence:

Asp-Val-Lys-Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr-Val-Leu-Val.

64. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 13.
65. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 22.
66. Isolated tumor rejection antigen.
67. Isolated human tumor rejection antigen.
68. Isolated tumor rejection antigen of claim 66, having amino acid sequence of figure 10.
69. Isolated tumor rejection antigen of claim 66, wherein said tumor rejection antigen is antigen E.
70. Isolated tumor rejection antigen of claim 66, wherein said tumor rejection antigen is antigen F.
71. Vaccine useful in treating a subject afflicted with a cancerous condition comprising a tumor rejection antigen precursor (TRAP0 of claim 58 which provokes an immune response when administered to a subject.
72. Vaccine of claim 71, wherein said TRAP is a human TRAP.

57. Expression system useful in transfecting a cell, comprising (i) a first vector containing a nucleic acid molecule which codes for a tumor rejection antigen precursor, and (ii) a second vector selected from the group consisting of (a) a vector containing a nucleic acid molecule which codes for an MHC or HLA molecule which presents a tumor rejection antigen derived from said tumor rejection antigen precursor, and (b) a vector containing a nucleic acid sequence which codes for an interleukin.

58. Isolated tumor rejection antigen precursor.

59. Isolated human tumor rejection antigen precursor.

60. Isolated tumor rejection antigen precursor of claim 58, wherein said precursor is mage-1.

61. Isolated tumor rejection antigen precursor of claim 58, wherein said precursor is a precursor for antigen F.

62. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 2.

63. Isolated tumor rejection antigen precursor coded for by the nucleic acid molecule of claim 12.

50. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 18 operably linked to a promoter.
51. Expression vector useful in transfecting a cell comprising the isolated nucleic acid molecule of claim 22 operably linked to a promoter.
52. The expression vector of claim 44, further comprising a nucleic acid molecule which codes for an MHC or HLA.
53. The expression vector of claim 44, further comprising a nucleic acid molecule which codes for a cytokine.
54. The expression vector of claim 53, wherein said cytokine is an interleukin.
55. The expression vector of claim 54, wherein said interleukin is IL-2.
56. The expression vector of claim 54, wherein said interleukin is IL-4.

31. Biologically pure culture of a highly transfectable cell line derived from a parent cell line which expresses at least one P815 tumor antigen, wherein said highly transfectable cell line does not express any of P815 tumor antigens A, B and C.
32. Biologically pure cell line of claim 31, comprising cell line PO.HTR.
33. Biologically pure culture of a cell line of claim 27, wherein said tumor rejection antigen precursor is a human tumor antigen precursor.
34. Biologically pure culture of a cell line of claim 33, wherein said human tumor antigen precursor is found in melanoma cells.